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Imaging based agglutination measurement of magnetic micro-particles on a Lab-on-a-Disc platform

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In this work we present a magnetic micro beads based agglutination assay on a centrifugal microfluidic platform. An imaging based method is used to quantify bead agglutination and measure the concentration of analyte in solution.

Low-cost and fast point-of-care (POC) testing has been of central interest in the last decade, both in the microfluidics research community as well as for industry. Among the large range of microfluidic platforms available (e.g. pressure driven, electroosmotic or capillary driven), the centrifugal platform is one of the most suitable ones for POC testing due to its robust working principle, low complexity and straight-forward separation of cells [1]. One of the challenges however is to use one readout method to perform multiple assays on the same platform e.g. immuno assays and cell based assays and thus reducing complexity and cost.

This work is the first step towards performing an imaging based readout of an agglutination assay on a centrifugal platform. While agglutination assays can typically be easily read out using absorbance based methods, the here presented approach is more versatile since an imaging based method could for example also be used to identify and count cells. Indeed, the image acquisition system used in this work has been developed for 3D cell classification (Unisensor A/S, Denmark) and is commercially available.

All microfluidic chips used in this work have been manufactured in PMMA using a CO₂ laser and bonded using pressure sensitive adhesive (Fig. 1). Experiments have been performed on a custom made centrifugal spin stand and image acquisition for cluster analysis has been performed using a standard microscope or the Thor detection system (Unisensor, Denmark) which has been integrated in the test stand (Fig. 2). All agglutination assays have been carried out using magnetic micro particles with diameters of 1 micrometer (coated with streptavidin) or 2.8 micrometer (coated with anti-CRP IgG). In order to demonstrate the assay, beads have been incubated with either biotinilated anti-streptavidin IgG or CRP for 10 min or 30 min, respectively and exposed to an external magnetic field to accelerate cluster formation. Subsequently beads have been redistributed by shaking and images have been acquired.

In order to demonstrate the technology, experiments have been performed detecting biotinilated anti-streptavidin IgG with streptavidin coated beads. The images of the clustered beads have been acquired using a standard microscope and were analyzed in a custom made Matlab program (Fig. 3). This demonstrates that this approach is capable of quantifying different degrees of agglutination depending on the analyte concentration. Subsequently experiments to detect CRP have been performed. For these experiments the slide chips shown in Fig. 1b have been used and read out using the Thor system. The results of these experiments are shown in Fig. 4.

In summary we demonstrate two agglutination based assays on a centrifugal platform which are read out using an imaging based technology. Currently work is focused on optimizing the CRP assay and integrating sample treatment on disc to perform CRP detection in blood.

Word Count: 492

References

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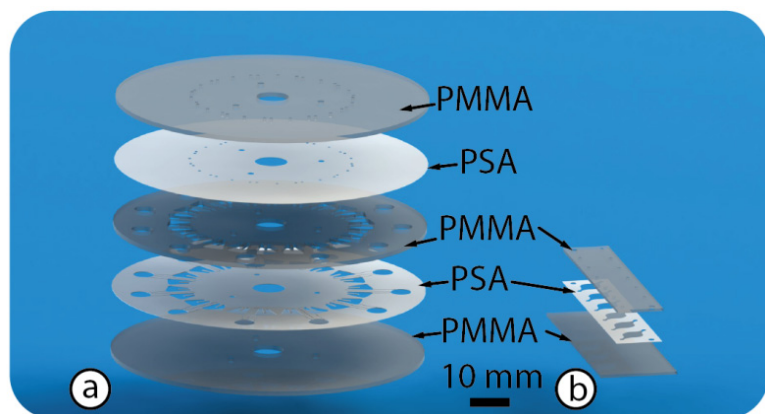


Figure 1: Assembly of the microfluidic disc (a) containing 11 independent test structures. Illustration (b) shows the slide chip with 6 independent assay chambers used with the Thor setup. PMMA = Poly(methyl methacrylate), PSA = Pressure Sensitive Adhesive

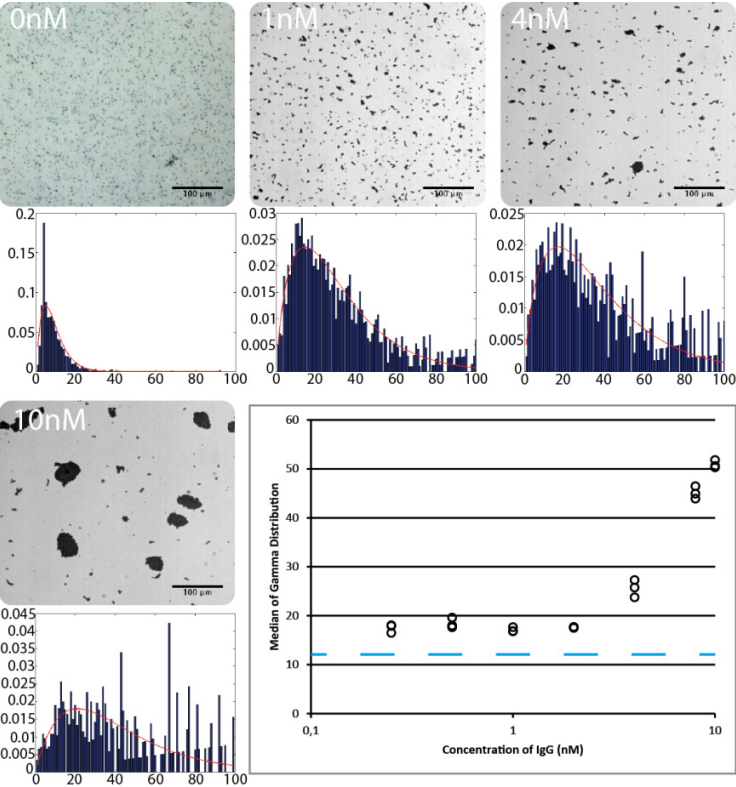


Figure 3: Results of agglutination assays using streptavidin coated magnetic beads with a diameter of 1 micrometer to detect biotinylated anti-streptavidin in PBS buffer. The images show the cluster formation as a result of the presence of the analyte in the buffer and the corresponding histograms of the size distributions. The graph shows the mean of the size distribution as a function of the analyte concentration. The dashed blue line indicates the blank value. Scale bars are 100 micrometer.

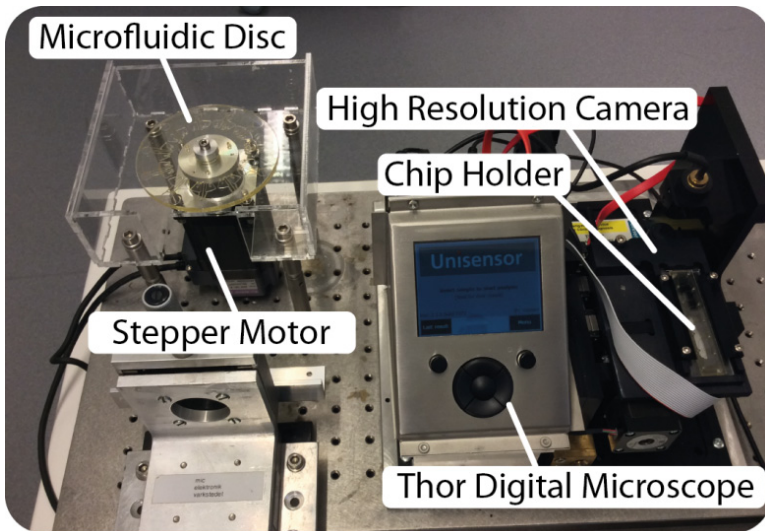


Figure 2: Test stand for spinning the microfluidic discs. The digital microscope used for reading out the slide chips is shown on the right hand side.

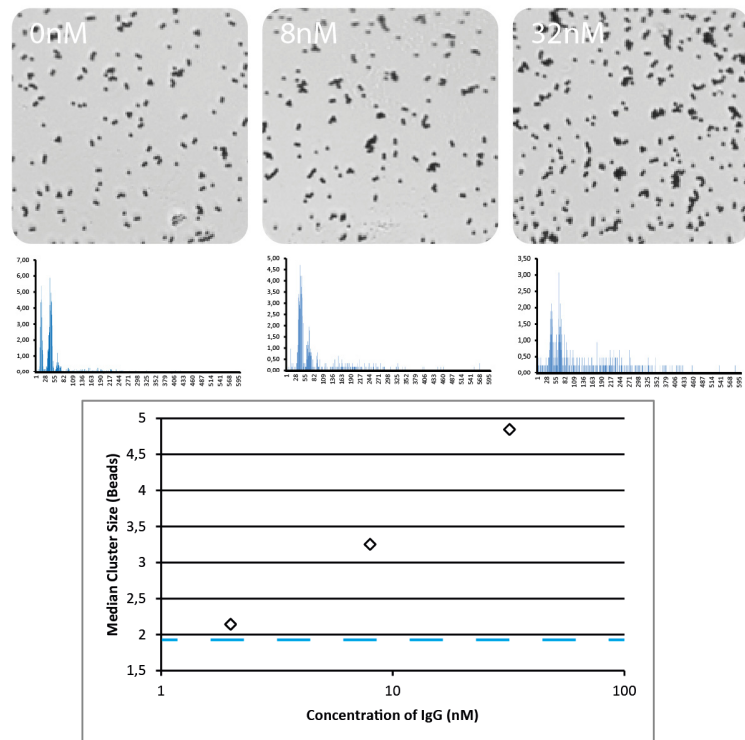


Figure 4: Preliminary results of the experiments to detect CRP in PBS buffer using magnetic beads with a diameter of 2.8 micrometer. Beads have been functionalized with anti-CRP IgG and form visible clusters in the presence of CRP. The graph shows the mean cluster size as a function of the CRP concentration. The dashed blue lines indicates the blank value.